SUMMARY

Although the unit of sensitizer is more easily destroyed by exposure to ultraviolet light than the unit of alexin, the difference is not real, since the concentration of serum proteins is enormously different. In dilutions more nearly comparable alexin is more quickly destroyed than sensitzer.

The aromatic amino-acids, tyrosin and phenylalanin, proteins containing these amino acids and certain substances belonging to the aromatic series show marked protective action for alexin and sensitizer against the action of ultraviolet rays.

Since destruction cannot take place without absorption it is possible that the difference in susceptibility shown by alexin and sensitizer toward ultraviolet light is due to a difference in aromatic amino-acid content.

THE CAUSE OF ABORTION IN MARES

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Studies of the etiology of abortion in mares have been made from time to time and reports from different localities assign as the probable cause an organism of the paratyphoid group. The work now presented was begun to determine whether there could be any relationship between abortions occurring among mares and an outbreak of influenza which was prevalent at this time on the farm. No such relationship could be established, but an organism has been isolated which is closely related if not identical with the one described by Meyer, Good and others. So far as known, this is the first report of the isolation of this organism in the west. Smith and Kilbourne[1] in 1893 isolated an organism resembling B. suipestifer from an aborting mare in Pennsylvania. In 1897 Lignieres described an organism of the hog cholera group in an outbreak of epizootic abortion in mares in France and South America. In 1912 Good,[2] in Kentucky, isolated from the placenta and uterus of aborting mares and from the internal organs of aborted foals an organism of the intermediate subgroup of the colon-typhoid group, with which he produced abortion in animals including mares. For this organism he proposes the name B. abortivo-equinus. Van Heelsbergen[3] and de Jong[4] report the occurrence of abortion in mares in Holland, and the isolation of an organism resembling B. paratyphosus B. With this organism they suceeded in producing abortion in small animals and also in the mare and cow. Meyer showed that the agent of epizootic abortion of mares in Pennsylvania is a bacillus of the paratyphoid-enteritidis group and proposed the name of B. abortus equi.

Dec. 24, 1916, two mares were brought to the clinic, one (1088 A) having aborted during the night and the other (1088 B) showing restlessness and

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[1] Bureau of Animal Industry, Bulletin 3, 1893, p. 53.

[2] Ky. Agr. Exp. Station, Bulletin 165, 1912; Proc. U. S. Live Stock Sanitary Association, 1911, p. 114; Jour. Infect. Dis., 1913, 13, p. 53.

[3] Centralbl. f. Bakteriol. I, O., 1913, 72, p. 38; Ztschr. f. Infektionskrank. der Haustiere, 1914, 16, p. 195.

[4] Centralbl. f. Bakteriol., I, O., 1912, 67, p. 148.

premonitory symptoms of abortion. Examination of the latter revealed a foal in anterior presentation with forelimbs retained by flexion at the elbow. The limbs were extended and traction exerted and delivery accomplished. The mares were isolated and all precautions taken against a spread of infection. The mares were from a winter pasture in which 8 others had been confined during the late fall and early winter. They had been fed cane with grain twice daily. Dec. 25 a third mare (1089) aborted in the same pasture; Dec. 27 four others; on Dec. 28 one other, and on Jan. 1 another. The following table shows stages of pregnancy:

TABLE 1

STAGES OF PREGNANCY IN MARES

Case	Bred	Aborted	Stage of Pregnancy
1,088A	May 15, 1916	Dec. 24, 1917	223 days
1,088B	May 17, 1916	Dec. 24, 1917	221 days
1,089	July 14, 1916	Dec. 25, 1917	164 days
1.091	July 1, 1916	Dec. 27, 1917	179 days
1,092	June 3, 1916	Dec. 27, 1917	207 days
1,093	Unknown	Dec. 27, 1917	
1,094	May 27, 1916	Dec. 27, 1917	214 days
1,095	May 27, 1916	Dec. 28, 1917	215 days
1,096	May 25, 1916	Jan. 1, 1917	221 days

Necropsy of the fetus, mare 1088 A, revealed the following: The fetal membranes were of a dark brownish-red color, rich in blood vessels and highly injected. The chorion and umbilical cord edematous and much thickened by serous infiltration. The skin normal, but subcutaneous tissue slightly infiltrated with a serous fluid. Over the surface of the placenta and the umbilical vessels was a fibrous exudate with papillary elevations of a grayish-white color, a condition observed in the membranes of all of the aborted foals, as well as in the fetuses of other species of animals aborted by injection of cultures from this fetus. Meyer and Boerner[5] report a similar appearance of the chorion and amnion in one or more of the cases which came under their observation. The organism isolated was studied with results as follows:

Morphology.—The organism from the aborted fetus is a short, thick, ovoid rod with ends rounded, and a tendency to bipolar staining in the smears. The size varies from 0.2 to 0.5 mikrons by 0.5 to 1.5 mikrons. No uniformity of arrangement is observed, the organism usually lying singly, occasionally in pairs and rarely in short chains. It stains well with the ordinary stains and is gram-negative. In a hanging drop it is actively motile.

Isolation and Culture.—Isolation can be made directly from the fetal blood stream and the internal organs by plating or direct inoculation on plain agar, Endo agar or Drigalski medium.

Agar Streak.—After from 12 to 24 hours a grayish-white, glistening, abundant growth appears. After from 24 to 48 hours the growth becomes membranous and wrinkled and extends over the water of condensation as a dry appearing, brittle film. The water of condensation becomes turbid. Observations of old cultures show that the membranous condition persists.

Agar Plates.—Small, round, slightly elevated, glistening colonies, later showing a finely granular structure develop rapidly. These become dry, membranous and tough, and entire colonies may be moved along the surface of the agar

[5] Jour. Med. Research, 1913, 29, p. 325.

with the platinum needle. The colonies in thickly sowed cultures remain pin point in size. If well separated they attain a size up to 4 or 5 mm. They remain discrete as a rule.

Gelatin.—Stab cultures are filiform. The surface growth is rapid and abundant, no liquefaction after 12 days' incubation.

Plain Broth.—After 24 hours the medium becomes uniformly turbid. After 48 hours a pellicle begins to develop which later settles to the bottom.

Milk.—After 10 days milk shows no coagulation, but changes to a yellow color. Reaction to litmus is alkaline.

Barsiekow 1 (Litmus-nutrose-dextrose).—Coagulation with marked acid production after 24 hours.

Barsiekow 2 (Litmus-nutrose-lactose).—This medium remains normal.

Hetsch (Litmus-nutrose-mannite).—The medium after 24 hours is milky. After 48 hours coagulation of the casein occurs, the color changes from rose to light pink and 40 to 60% of gas is formed after 72 hours.

Löffler 1.—Turbidity and gas formation, manifested by a greenish froth on the surface of the medium occur after 24 hours. Turbidity increases and after 48 hours a yellowish green precipitate of nutrose casein forms and gradually settles along the sides and at the bottom of the tube. A gradual clearing of the fluid at the upper part of the tube occurs.

Löffler 2.—For 24 hours the medium is unchanged. After 48 hours the medium loses its green color, rapidly changing from a dirty yellow to gray, and finally is colorless.

Litmus Whey.—After 24 hours the medium is dark red, changing to dark rose after 48 hours, to normal color after 72 hours, and to dark blue after 192 hours.

Neutral Red.—In 24 hours the medium begins to decolorize, gas formation marked by bubbles occurs and the fluorescence develops. Decolorization begins at the bottom, and after 192 hours only a narrow zone of red is left at the top.

Orcein Agar.—No change in this medium is noted.

Dextrose Broth.—Evolution of gas is rapid and in 72 hours from 48-50% of gas is present. The reaction is slightly acid.

Lactose Broth.—Growth is luxuriant in this medium but no gas is produced.

Dunham's Peptone.—No indol is produced. Pellicle formation is not so marked as on plain bouillon.

Drigalski Medium.—Colonies are blue.

Endo Agar.—White, disk-shaped colonies resembling those on plain agar develop after 18-24 hours.

Inoculation Experiments.—The culture used was isolated from the umbilical vein of the fetus of mare 1088 A, Dec. 24, 1916. Rabbit 150 was bred March 12, 1916; on April 2, two loopfuls (4 mg.) of a 24-hour agar culture in 2 c c salt solution were injected intraperitoneally. April 4 mother rabbit aborted 3 dead fetuses and was killed; the point of injection was congested, the peritoneum slightly congested and covered with a purulent exudate. The external surface of the uterus was congested and hemorrhagic, internally slightly congested and covered with a thin weblike fibrinous exudate studded with granular elevations of a grayish-white color. The kidneys were slightly congested. The spleen was covered with thin fibrinous exudate.

The smears from peritoneal fluid, blood, uterine exudate, stained with methylene blue, all showed the characteristic organism. Pure cultures were obtained on agar; cultures were obtained also from an aborted fetus.

Mare 1.—This mare was bred July 4, 1916; on April 5, 1917, an intravenous injection of two loopfuls (4 mg.) of a 24-hour agar culture of the organism isolated from the uterus of the rabbit on April 4 was made. The mare aborted during the night of April 16. No premonitory symptoms were observed except the relaxation of the sacro-sciatic ligament, first noticeable on April 9, and a slight vaginal discharge on the evening of April 16. There were no noticeable disturbances of the temperature from April 4 to 24.

The fetal membranes were intact and the fetus was dead when discovered. Smears from the blood of the umbilical vein, the liver, the intestines, spleen, lungs and heart all showed organisms. The changes were as follows: Umbilical vessels covered with a fibrinous exudate on which were numerous papillary elevations of the color and appearance as in the uterus of rabbit 150. Maternal placenta covered with a slimy exudate on the internal surface; liver, slightly congested, a few hemorrhages; intestines, normal; lungs and heart, a few small hemorrhages; thymus, congested; spleen, congested and hemorrhagic. From the fetus cultures of the organism were obtained from the organs and blood.

Sow 1.—This was a cholera immune sow in about the twelfth week of pregnancy. May 5 she was injected intravenously with 2 loopfuls (4 mg.) of 24-hour agar culture in 2 c c of salt solution of the organism from the fetus of the aborted rabbit. On May 7 she aborted one pig, followed within a few hours by four others. The first pig showed the skin everywhere hemorrhagic; the peritoneal cavity contained a large quantity of serous fluid in which were the characteristic organisms; the layers of the peritoneum were hemorrhagic; the spleen was congested, and in the other organs there were many minute hemorrhages.

On the fetal placenta the characteristic granules seen in other placenta of aborted animals were present. The organism was recovered from this pig.

Guinea-Pig 192.—Inoculated intraperitoneally, Oct. 18, 1917, with 1 loopful of a 24-hour agar culture of bacillus from fetus of mare in 3 c c of salt solution. Since isolation, a period of 6 months, the organism had been transferred a number of times, being carried as a stock culture but had not been passed through any animals. Oct. 22, an edematous swelling appeared at the point of injection; the next day the animal had 3 dead young, partly haired, the skin of which showed a few hemorrhages; the subcutis and the thymus gland were congested; other organs apparently normal. Inoculations on agar from the blood, liver, and spleen were negative. The mother pig was killed a few hours after the abortion. A hard infiltrated area was present around the point of inoculation; slight inflammation of peritoneum in region of the uterus; inguinal glands on the side of inoculation enlarged; uterine walls thick, congested, lining deeply injected; liver mottled, brown; spleen showed numerous abscesses; other organs normal. The characteristic gram-negative bacillus was present in the blood, spleen, liver and uterus.

Agglutination tests by other investigators using as antigen organisms that are apparently the same as the one under consideration have shown that normal horse serum agglutinates in dilutions of from 1:40 to 1:300; my observations indicate that the lower titer is the commoner.

The agglutinating titers of serum from mares that have aborted from natural infection, as recorded by Meyer, may show a variation of from 1:1,200 to 1:2,500. DeJong[4] records 1:1,000 as the highest titer of serum from mares that had aborted from a natural infection. My own observations show generally a lower titer (Table 2).

TABLE 2

AGGLUTINATION TESTS

Antigen	Titer		
	Mare 1,092 7 Days after Abortion	Mare 1,091 10 Days after Abortion	Mare 1,088A 101 Days after Abortion
Fetus 1,088A	640	320	320
Kentucky (Piatt)	1,280	1,280	630
Kentucky (Sis)	1,280	640	1,280
Pennsylvania (Reichel)	640	320	320

The experimentally infected mare that aborted gave titers as follows:

Before abortion, Pennsylvania (Reichel) 1:80
Three days after abortion, culture from fetus of mare 1088 A 1:1,280
Three days after abortion, Kentucky (Piatt) 1:1,280
Three days after abortion, Kentucky (Sis) 1:1,280
Three days after abortion, culture from own fetus 1:1,280
Three days after abortion, Pennsylvania (Reichel) 1:640
Eight days after abortion, culture from fetus of mare 1088 A 1:1,280
Eight days after abortion, culture from own fetus 1:640
Eight days after abortion, Pennsylvania (Reichel) 1:640

Serum from the sow drawn eight days after abortion showed an agglutinating titer of 1:2,500 for the strain of organism inoculated as compared with a titer of 1:20 of normal pig serum for the same organism.

Agglutination tests with highly immune serum from rabbits systematically immunized against the organism causing abortion present interesting evidence of a close relationship between this organism and organisms of the intermediate sub-group, Table 3.

TABLE 3

AGGLUTINATION TESTS WITH IMMUNE RABBIT SERUM

Antigen	Titer		
	Serum of Rabbit Immunized with Bacillus from Fetus, Mare 1,088A	Serum of Rabbit Immunized with Organism from Fetus, Mare 1	Serum of Rabbit Immunized with Kentucky (Piatt) Strain
Culture from fetus 1,088A	5,120	10,240	2,560
Kentucky (Piatt)	5,120	10,240	2,560
Pennsylvania (Reichel)	2,560	10,240	2,560
Culture, Mare 1	2,560	5,120	2,560
B. paratyphosus B.	640	640	40
B. enteritidis	160	160	80
B. suipestifer	160	160	160
B. paratyphosus A	0	0	0
B. coli	0	0	0
B. paracoli	0	0	0
B. typhoses	0	0	0

Agglutination reactions using serum of rabbits immune to various organisms of the typhoid-enteritidis group against the different strains of B. abortivo-equinus gave results (table 4) that indicate relationships.

TABLE 4

AGGLUTINATION TESTS WITH TYPHOID-ENTERITIDIS SERUM (RABBIT)

Antigen	Titer				
	Antiparacoli Serum	Antityphimurium Serum	Antienteritidis Serum	Autiparatyphosus B Serum	Antiparatyphosus A Serum
B. paracoli	1,280	—	—	—	—
Fetus, Mare 1	0	320	320	160	320
Pennsylvania (Reichel)	0	640	320	160	640
Kentucky (Piatt)	0	320	160	160	640
Kentucky (Sis)	0	640	320	160	640
B. typhimurium	—	1,280	—	—	—
B. enteritidis	—	—	2,560	—	—
B. paratyphosus B	—	—	—	5,120	—
B. paratyphosus A	—	—	—	—	5,120

Meyer and Boerner[5] obtained these results with the organism isolated by them and serum from animals immune to members of the paratyphoid group: B. paratyphosus A, 1:20; B. paratyphosus B, 1:100; B. enteritidis 1:80. They also found that three paratyphoid strains were agglutinated by serum from rabbits immune to B. abortivo-equinus in dilutions from 1:20 to 1:100. B. enteritidis was agglutinated by the same serum in dilutions somewhat higher than the paratyphoid strains. A suipestifer strain was agglutinated at 1:600. They conclude from their results that the organism can be separated serologically from the main representatives of the paratyphoid-enteritidis group.

Lautenbach[6] concludes on the basis of agglutination tests that the organism must be classed in the group of the hog cholera bacilli, and that it stands nearest B. paratyphosus A.

De Jong[4] found that serum from two mares which had aborted agglutinated the organism found by him in dilutions as high as 1:1,000, while normal serum agglutinated only up to 1:300. A mare injected by him aborted on the 11th day and the organism recovered from the placenta was agglutinated by the serum from this animal in dilution of 1:1,000. A cow injected at the same time aborted in 15 days, and the organism recovered from the placenta was agglutinated by the serum of the cow in dilution of 1:600, while the titer of normal cow serum was 1:100. A mare fed a culture aborted and the bacillus was

[6] Centralbl. f. Bakteriol., I, O., 1913, 71, p. 349.

found in the fetus. The serum of this animal agglutinated the organism in dilution of 1 : 1,500 while normal horse serum gave a titer of 1 : 300. His conclusion is that the organism from aborted foals belongs to the paratyphosus B. enteritidis group, and that it is agglutinated in much higher dilution by the serum from aborted mares than by normal serum.

Good,[2] with serum from guinea-pigs immune to various members of the paratyphoid organisms, obtained little or no agglutination of the organism isolated by him from aborting mares.

Van Heelsbergen,[3] using the serum of an artificially immunized mare with an agglutinating titer of 1 : 10,000, found that three different strains of B. suipestifer were agglutinated in dilutions no higher than 1 : 100. Three strains of B. enteritidis were not agglutinated above 1 : 300. Five strains of paratyphosus B. were agglutinated in dilutions of from 1 : 100 to 1 : 400, while B. typhi-murium was agglutinated in dilution of 1 : 2,400. His conclusion is that this behavior differentiates the bacillus not only from the swine pest organism, but also from the other different paratyphoid B.-enteritidis bacteria. He holds that up to the present time no single paratyphoid B.-enteritidis bacillus has been found which is agglutinated by a high dilution of the immune abortion serum that he prepared in the same manner as the specific strain, so that as a tentative test agglutination is the best method for the identification of the abortion bacillus. The only one of the paratyphoid B.-enteritidis group which is agglutinated in high dilution by abortion serum is B. typhi-murium of Loeffler, which was agglutinated in dilution of 1 : 2,400.

My results with agglutination tests more nearly correspond to those of Van Heelsbergen than to those of other investigators.

SUMMARY

From an outbreak of abortion in Iowa there was isolated an organism of the paratyphoid-enteritidis group, which in cultural and morphologic characters, and in serologic reactions is apparently the same as the organism studied by Good,[2] Meyer,[5] and others, and variously named by them B. abortivo-equinus, B. abortus-equi, etc. The dry, brittle, membranous growth on slanted agar which was observed by these investigators was present in the cultures isolated in this outbreak, and this peculiarity is of great value in identification of the organism.

With this organism abortion was produced in the rabbit, guinea-pig, sow and mare by intraperitoneal or intravenous injection of minute doses in 2, 6, 2, and 11 days, respectively. Feeding and intravaginal introduction of the organism did not result in abortion in any animals thus treated.

The organism is agglutinated by immune serum for the organisms of paratyphoid-enteritidis group in fairly high dilutions. Serum of rabbits immune to this organism also agglutinate B. suipestifer, B. enteritidis (Gaertner) and B. paratyphosus A and B in dilutions high enough to indicate a close serologic relationship to these organisms.